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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Mike FARWICK, et al.

Serial No. : 10/098,626

Filed: March 18, 2002

Examiner: To Be Assigned

Group Art Unit: 1623

For : PROCESS FOR THE PREPARATION OF L-AMINO ACIDS BY USING CORYNEFORM BACTERIA

RESPONSE AND AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notice of Omitted Items in a Non-Provisional Application mailed August 1, 2002, please amend the application as follows.

IN THE SPECIFICATION:

Delete pages 28-34 and substitute new pages 28-32 submitted herewith.

Remarks

Applicants have correctly re-numbered the pages of the specification in the above-identified patent application. No new matter has been added.

If any fees are due in connection with this filing, such as fees under 37 C.F.R. §§ 1.16 or 1.17, the Commissioner is authorized to charge the fees to SGR Deposit Account No. 02-4300; Order No. 032301.269. Similarly, please credit any overpayment to SGR Deposit Account No. 02-4300.

Respectfully submitted,,
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What is claimed is:

1. A polynucleotide isolated from coryneform bacteria, containing elongated polynucleotide sequences coding for 1-phosphofructokinase and/or 6-phosphofructokinase, wherein said sequences are each elongated in front of the start codon and behind the stop codon of the gene, in each instance by up to about 700 base-pairs.

2. Isolated polynucleotide according to Claim 1, containing (a) polynucleotide sequence(s) coding for 1-phosphofructokinase and/or 6-phosphofructokinase, wherein said sequence(s) are elongated in front of the start codon and behind the stop codon of the gene, in each instance by up to about 700 base-pairs, the elongated amino-acid sequences being represented in SEQ ID No. 3 for the 1-phosphofructokinase gene and in SEQ ID No. 1 for the 6-phosphofructokinase gene and the elongations in comparison with the sequences known from the state of the art consisting in SEQ ID No. 3 of base-pairs 1 to 508 and 1684 to 2234 and in SEQ ID No. 1 of base-pairs 1 to 531 and 1621 to 2160.

3. A process for the fermentative preparation of L-amino acids, in particular L-lysine, wherein the following steps are implemented:

   a) fermentation of the coryneform bacteria producing the desired L-amino acid, in which at least the gene coding for 6-phosphofructokinase and/or the gene coding for 1-phosphofructokinase are/is attenuated,

   b) enrichment of the desired product in the medium or in the cells of the bacteria, and

   c) isolation of the desired L-amino acid, whereby constituents of the fermentation broth and/or of

the biomass optionally remain in the end product in proportions or in their total quantities.

4. Process according to Claim 3, wherein coryneform bacteria are employed in which the attenuation is achieved by using the polynucleotide sequences that are elongated in front of the start codon and behind the stop codon of the respective gene by, in each instance, 300 to 800 base-pairs.

5. Process according to Claim 4, wherein coryneform bacteria are employed in which the attenuation is achieved by using the polynucleotide sequences that are elongated in front of the start codon and behind the stop codon of the gene, in each instance by about 700 base-pairs, the elongated nucleotide sequences being represented in SEQ ID No. 3 for the 1-phosphofructokinase gene and in SEQ ID No. 1 for the 6-phosphofructokinase gene and the elongations in SEQ ID No. 3 in comparison with the sequence known from the state of the art consisting of base-pairs 1 to 508 and 1684 to 2234 and in SEQ ID No. 1 in comparison with the sequence known from the state of the art consisting of base-pairs 1 to 531 and 1621 to 2160.

6. Process according to Claim 3, wherein bacteria are employed in which, in addition, further genes of the biosynthetic pathway of the desired L-amino acid are enhanced.

7. Process according to Claim 3, wherein bacteria are employed in which the metabolic pathways that diminish the formation of the desired L-amino acid are at least partially switched off.

8. Process according to Claim 3, wherein the expression of the polynucleotide(s) that codes/code for 6-phosphofructokinase and/or for 1-phosphofructokinase is diminished.

9. Process according to Claim 3, wherein the catalytic properties of the polypeptide(s) (enzyme protein(s)) for which the polynucleotide(s) from SEQ ID NO. 1 and SEQ ID No. 3 codes/code are reduced.

10. Process according to Claim 3, wherein for the preparation of L-lysine coryneform micro-organisms are fermented in which simultaneously one or more of the genes selected from the group comprising

10.1 the gene lysC coding for a feedback-resistant aspartate kinase,

10.2 the gene dapA coding for dihydrodipicolinate synthase,

10.3 the gene gap coding for glyceraldehyde-3-phosphate dehydrogenase,

10.4 the gene pyc coding for pyruvate carboxylase,

10.5 the gene mqo coding for malate:quinone oxidoreductase,

10.6 the gene zwf coding for glucose-6-phosphate dehydrogenase,

10.7 simultaneously the gene lysE coding for lysine export,

10.8 the gene zwa1 coding for the zwa1 protein,

10.9 the gene tpi coding for triosephosphate isomerase, and

10.10 the gene pgk coding for 3-phosphoglycerate kinase

is/are enhanced, in particular overexpressed.

11. Process according to Claim 3, wherein with a view to preparing L-amino acids coryneform micro-organisms are fermented in which simultaneously one or more of the genes selected from the group comprising

11.1 the pck gene coding for phosphoenolpyruvate carboxykinase,

11.2 the pgi gene coding for glucose-6-phosphate isomerase,

11.3 the gene poxB coding for pyruvate oxidase,

11.4 the gene fda coding for fructose bisphosphate aldolase, and

11.5 the gene zwa2 coding for the zwa2 protein

is/are attenuated.

12. Process according to one or more of Claims 3 to 11, wherein micro-organisms of the species Corynebacterium glutamicum are employed.

13. Coryneform bacteria in which at least the gene coding for 6-phosphofructokinase and/or the gene coding for 1-phosphofructokinase are/is present in attenuated form.

14. Escherichia coli strain DH5alphamcr/pXK99EmobpfkB (= DH5αmcr/ pXK99EmobpfkB), deposited as DSM 14741 in the Deutschen Sammlung für Mikroorganismen und Zellkulturen (German Collection of Micro-Organisms and Cell Cultures), Braunschweig, Germany.

Abstract

The invention relates to a process for the preparation of L-amino acids, wherein the following steps are implemented:

a) fermentation of the coryneform bacteria producing the desired L-amino acid, in which at least the gene coding for 6-phosphofructokinase and/or the gene coding for 1-phosphofructokinase are/is attenuated,

b) enrichment of the desired L-amino acid in the medium or in the cells of the bacteria, and

c) isolation of the L-amino acid,

and optionally bacteria are employed in which, in addition, further genes of the biosynthetic pathway of the desired L-amino acid are enhanced, or bacteria are employed in which the metabolic pathways that diminish the formation of the desired L-amino acid are at least partly switched off.